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High pressure processing of meat: possible role of myofibrillar protein interactions and cathepsin activity

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Abstract

Background: The research of high pressure (HP) processing of meat based foods needs to address how pressure affects protein interactions, aggregation and/or gelation. The understanding of the gel forming properties of myofibrillar components is fundamental for the development of muscle based products (Chapleau *et al.*, 2004; Colmenero, 2002).

Object: The aim was to study the rheological properties of pork meat emulsion exposed to HP and the effect of HP on the aggregation state of myofibrillar proteins. To address the role of cathepsin in myofibrillar protein degradation the changes in the myofibrillar protein pattern and HP-induced change in activity of cathepsin B and L were investigated.

Results: In this study we showed that HP treatment of pork meat emulsion, ranging from 0.1 to 800 MPa, induced protein gel formation as shown by the increased Young's modulus (Fig.1). Analysis of SDS-PAGE gels of myofibrillar protein extract from HP treated meat showed that myofibrillar proteins form high molecular weight aggregates after HP treatment. Myofibrillar protein aggregates were stable in a reducing environment, suggesting that disulfide bonds are not the main molecular interactions responsible for these aggregations (Fig.2). Furthermore HP treatment caused an increase in cathepsin activity (Fig.3), probably due to disruption of the lysosomal membrane and leakage of enzymes, which subsequently affected the myofibrillar protein degradation pattern (Fig.4).

Conclusion: HP treatment affects the rheological properties of pork meat batters by inducing formation of protein gels. HP induced protein gels are suggested to be formed by high molecular weight myofibrillar protein aggregates and by peptides formed by lysosomal enzyme-induced cleavage of myofibrillar proteins.

Perspectives: The data presented suggest that the lysosomal enzymes play a potential role in pressure assisted gelation.

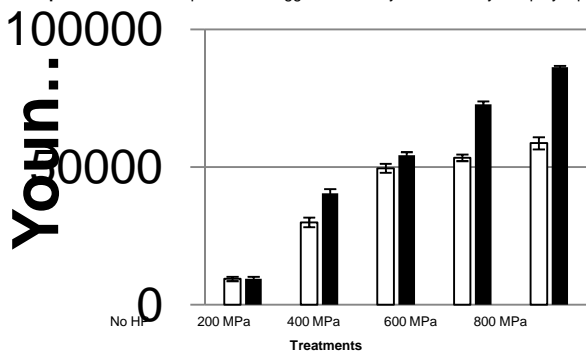


Fig.1 Rheological properties of HP-treated pork meat emulsions assessed by uniaxial compression. White bars represent pork meat emulsion treated at 5 °C and black bars represent meat emulsion treated at 40 °C. Samples were cylindrical in shape (60 mm in diameter and 20 mm in height). The sample was compressed at a constant crosshead velocity of 50 mm/min.

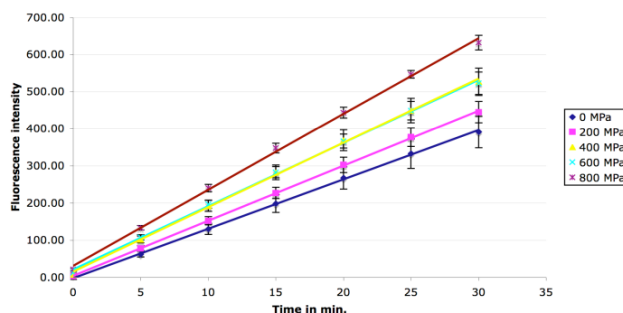


Fig.3 Cathepsin B+L activity affected by high pressure. Crude extract of pork meat HP treated (0.1,200,400,600 and 800 MPa) was performed in a sucrose solution (pH 7.2; 0.25 M sucrose, 50 mM Tris-HCL, 1 mM EDTA, 0.1 M KCL) at 4 °C. Enzymes activity was measured with a fluorescent synthetic peptide Z-Phe-Arg-MCA. The release of MCA induced by cathepsin hydrolysis of the Z-Phe-Arg-MCA substrate was monitored over a period of time of 30 min.

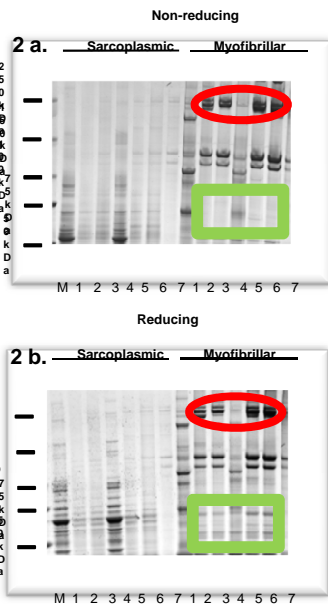


Fig.2 SDS-PAGE patterns of sarcoplasmic and myofibrillar proteins fractions. Sarcoplasmic and myofibrillar protein extracts were prepared from fresh pork meat, minced and not treated (1), minced HP at 800 MPa and 40 °C (2,3) and frozen pork meat, minced not treated (4) minced HP at 800 MPa and 40 °C (5,6) and pork meat heat treated at 72 °C. Protein extract were loaded onto 3-8% Tris-Acetate gels in non-reducing (Fig.2a) and reducing condition (Fig.2b) and stained with coomassie blue reagent. In this study no changes in the sarcoplasmic protein patterns were observed. A marked decrease in sarcoplasmic protein solubility (in both reducing and non-reducing conditions) was observed, when pork meat was treated at 800 MPa and 40 °C (Fig. 2a and 2b). The myofibrillar protein fraction exposed to 800 MPa at 40 °C (samples 2,3,5 and 6) showed the formation of protein aggregates of a molecular weight higher than myosin (marked in red). HP treated myofibrillar proteins when loaded into a gel in reducing conditions still show high molecular weight aggregates (Fig.2b red circle) and they present the formation of low molecular weight bands (Fig.2b green square). We speculate that these bands are products of cathepsin catalyzed hydrolysis of myofibrillar proteins.

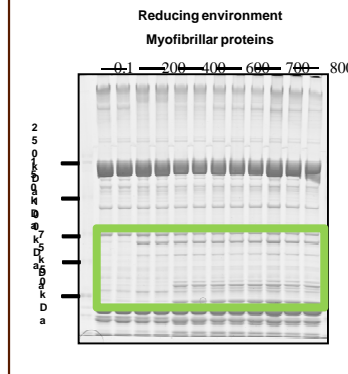


Fig.4 SDS-PAGE patterns of myofibrillar proteins. Myofibrillar protein extract were prepared from fresh pork meat minced and HP treated (0.1, 200, 400, 600 and 800 MPa) and loaded in 3-8% Tris-Acetate gels in reducing conditions. Myofibrillar protein band pattern changes in the molecular weight ranged between 45 and 100 kDa (green square) from 0.1 to 400 MPa. We speculate that the differences in band pattern are due to an increase of cathepsin leakage from the lysosomes due to high pressure treatment and subsequent protein degradation.

References

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